Nanostructured TiO_2 and TiO_2-Ag antimicrobial thin films

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ABSTRACT

Infection is one of the common clinical problems that cause high rates of death in various surgical operations. Coating the surgical instruments or implants with antibacterial properties is a useful method to reduce such infections during the operation period. The high incidence of infections caused by the use of implanted biomedical devices and environment in the hospital has a severe impact on the health of patients. However no comprehensive studies have been reported on active species of fungal and bacteria. In this present investigation, we have systematically studied the antifungal (against *Candida albicans* (MTCC-1637), *Candida tropicalis* (MTCC-184), *Candida parapsilosis* (MTCC-2509), *Candida glabrata* (MTCC 3019) and antibacterial properties of 4 species namely (against *Staphylococcus faecalis (NCIM-2604). Staphylococcus epidermidis (NCIM-2493), Staphylococcus aureus (NCIL-2122), Bacillus subtilis (NCIM-2549)* each using pure TiO_2 and TiO_2 with Ag as additive by using the simple and cost effective sol-gel process. The films have been deposited on glass and Si substrates and their structural, morphological properties have been investigated by employing standard spectroscopic techniques such as X-Ray Diffraction and Scanning Electron Microscopy, respectively. The test for antifungal and antibacterial has been carried out by using drop test method. It is evident from the results that with increasing Ag concentration the antimicrobial activity increases.

Keywords: *sol-gel process, thin films, X-ray diffraction, Scanning electron microscopy, microbialactivity*

1 INTRODUCTION

Recently there has been lot of investigation on the antibacterial coatings keeping in view of increasing bacterial resistance to antibiotics and antiseptics. Antibacterial activity of inorganic materials in particular metal oxides is of significant interest due to the need for infection control and rising antibiotic resistance. For other metal oxides, different mechanisms have been proposed to explain antibacterial activity. Antibacterial activity of different metal oxides has been assigned to the photocatalytic production of reactive oxygen species, but this mechanism has so far been overwhelmingly established for TiO_2 [1]. In general, antibacterial activity has been demonstrated both in the dark and under UV illumination, but the difference in the growth inhibition in these two cases was dependent both on the type of metal oxide, as well as the type of bacteria (different responses were observed for the same material for Gram-positive and Gram-negative bacteria). For some metal oxides, such as ZnO, exposure to ambient light and UV light resulted in higher activity compared to dark conditions, but the optimal illumination conditions may be particle size dependent [2-11].

Based on this observation, the present investigation deals with pure TiO_2 and TiO_2 with different concentrations of Ag as possible bioactive coatings with a biocidal capacity for antimicrobial application. In this work, we investigated the potential of sol-gel based coatings of pure TiO_2 and TiO_2 with different concentrations of Ag as additive. The annealed films are subjected for antifungal and antibacterial drop test method. These films can be of interest for number of potential applications, such as multifunctional coatings, as well as coating of nickel-plated tools, such as forceps, blades etc. For such applications, a basic deposition method, such as sol-gel, can be of significant interest since this process is simple, cost effective and easy to operate.

2 EXPERIMENTAL PROCEDURE

The The preparation and deposition of TiO_2 thin films by sol-gel spin coating process have been shown in the figure 1. Calculated quantity of titanium isopropoxide (Aldrich, 99.98 % purity) has been dissolved in 2-methoxy ethanol (99.98%) solvent along with acetyl-acetone as a complexing and chelating agent. 1.2 g of cetyl trimethyl ammonium bromide has been dissolved in 20 ml of 2-methoxy ethanol solvent in beaker and the contents were added drop wise to the titanium isopropoxide sol under vigorous stirring. The contents were stirred at room temperature for about 6 h. The molar ratio of titanium

isopropoxide, 2-methoxy ethanol and acetyl-acetone were 2.0:10.0:0.5. To the above contents calculated quantities of HNO_3 (1.2 ml) and of deionizing water (1.5 ml) have been added drop wise as a catalyst to increase the rate of reaction, and hydrolysis of the sol, respectively. Further the contents are refluxed at 70°C for 3h to complete the reaction and later cooled to room temperature. The contents were filtered using Wattman filter paper in order to remove any particulates formed during the reaction. The obtained stock solution is used for the deposition on to glass slides, quartz and Si substrates by spin coating process.

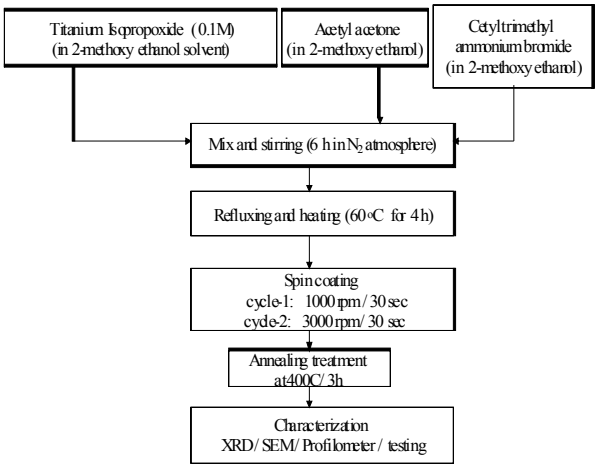

Fig 1: Flow chart for the preparation of nanostructured thin films of pure TiO_2 and TiO_2 with Ag as additives by cost effective sol-gel process

Antifungal activity of TiO_2:Ag thin films:
Sabouraud agar was prepared by using 40 g/L dextrose , 10 g/L peptone, 20 g/L agar1000 ml distilled water and the pH was adjusted to pH 5.6. The agar medium was sterilized in aquilots of 15ml at a pressure of 15 lbs for 15 min. This agar medium was transferred into sterilized Petri dishes in a laminar air flow unit and allowed them to solidify.100 µl of 24 hr fungal suspension grown in Sabouraud broth and standardized (0.5 McFarland standard) was added on each TiO_2 and Ag coated plates and kept in UV chamber for 4hrs. At a regular interval of 1hr the exposed plates were washed with sterile saline or distilled water into the prepared agar plates and culture was spread with a glass spreader to ensure equal distribution of the organism. The plates were incubated at 37^0C for 24 hrs and colony forming units (CFU) were counted. Simultaneously a positive and negative control was prepared.

Antibacterial activity of TiO_2:Ag thin films:
Nutrient agar medium was prepared by using peptone (5.0 g), beef extract (3.0 g), and sodium chloride (NaCl) (5.0 g) in 1000 ml distilled water and the pH were adjusted to 7.0 and agar (15.0 g) was added to the solution. The agar medium was sterilized in aquilots of 15ml at a pressure of 15 lbs for 15 min. This nutrient agar medium was transferred into sterilized Petri dishes in a laminar air flow unit and allowed them to solidify.100µl of 24 hr bacterial suspension grown in nutrient broth and standardized (0.5 McFarland standard) was added on each TiO_2 and Ag coated plates and kept in UV chamber for 4hrs. At a regular interval of 1hr the exposed plates were washed with sterile saline or distilled water into the prepared agar plates and culture was spread with a glass spreader to ensure equal distribution of the organism. The plates were incubated at 37^0C for 24 hrs and colony forming units (CFU) were counted. Simultaneously a positive and negative control was prepared. The preparation of the drop test for both antifungal and antibacterial test is shown in figure 2 (top and bottom), respectively.

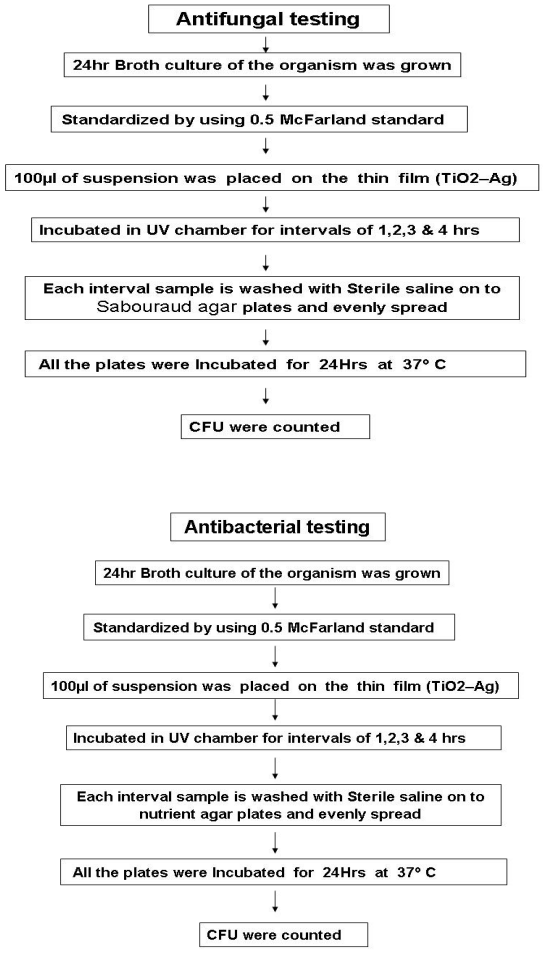

Fig 2: Antifungal species drop testing procedure (top) and Antibacterial species drop test (bottom) of nanostructured thin films of pure TiO_2 and TiO_2 with Ag (different concentrations) as additive by sol-gel process annealed at 400^oC for 1h in air ambient

3. Characterization

3.1 Morphological Characterization by SEM

Morphological studies on the treated films of pure TiO_2 and TiO_2 with different concentrations of Ag as additive have been carried out by employing high resolution scanning electron microscopy using a FEI Nova NANOSEM 230 instrument (model: ZIESS).

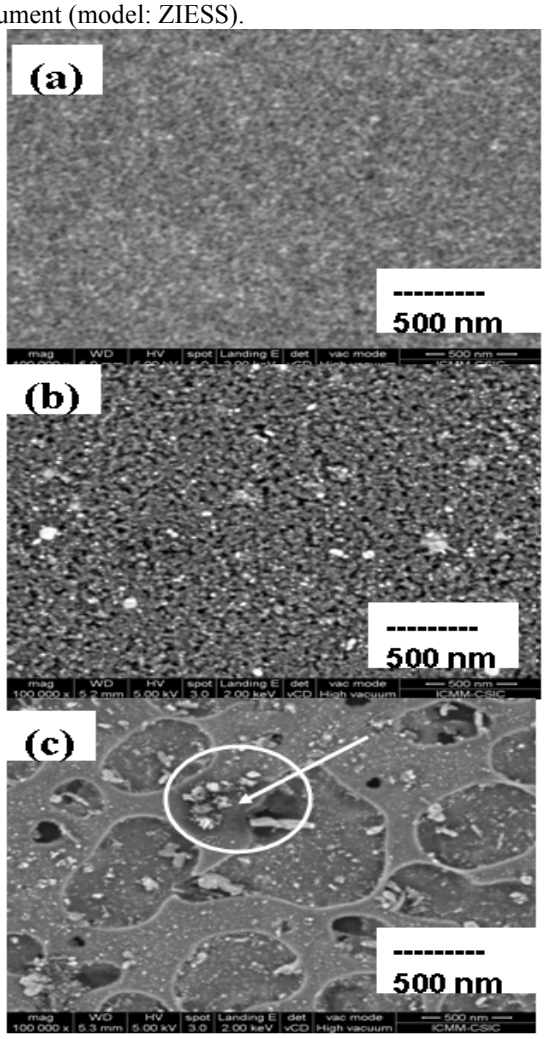

Fig 3: Scanning Electron Micrographs of nanostructured thin films of pure TiO_2 and TiO_2 with Ag as additives by cost effective sol-gel process annealed at 400°C for 1h in air ambient (a) Pure TiO_2 (b) TiO_2 with Ag (0.2 wt%) as additive (c) TiO_2 with Ag (0.8 wt%) as additive

The deposited and annealed films were uniform, homogeneous, crack free, and highly dense. This could be supported by the fact that the solvent 2-methoxy ethanol (low evaporation rate) used in the present investigation causing the treated films crack free. On the other hand, high magnification images revealed that the annealed films were mostly nanostructured with grain sizes ranging from 8 to 20 nm as shown in Figure 3a. No major difference in morphology as well as further change in the nanostructuring was observed for the films with low concentrations of Ag as additive as shown in Figure 3b. However films with highest concentration of Ag as additive have shown huge porous structures with agglomeration of Ag clusters in some areas as shown in the Figure 3c. The agglomeration of Ag in Figure 3c is represented by white circles.

4. Antimicrobial Testing

All the fungal species showed susceptibility to pure TiO_2 and TiO_2 Ag films namely, *Candida albicans* (CA) (MTCC-1637), *Candida tropicalis(CT)* (MTCC-184), *Candida parapsilosis (CP)* (MTCC-2509), *Candida glabrata (CG)* (MTCC 3019 as shown in the figure 4a-d, respectively. Among them *Candida parapsilosis* (MTCC-2509) showed much difference with pure TiO_2 and TiO_2 Ag films (Fig-4d). *Candida glabrata* (MTCC 3019) showed no much difference number of viable colonies with increase in concentration of Ag. It may be considered as resistant organism among the used species.

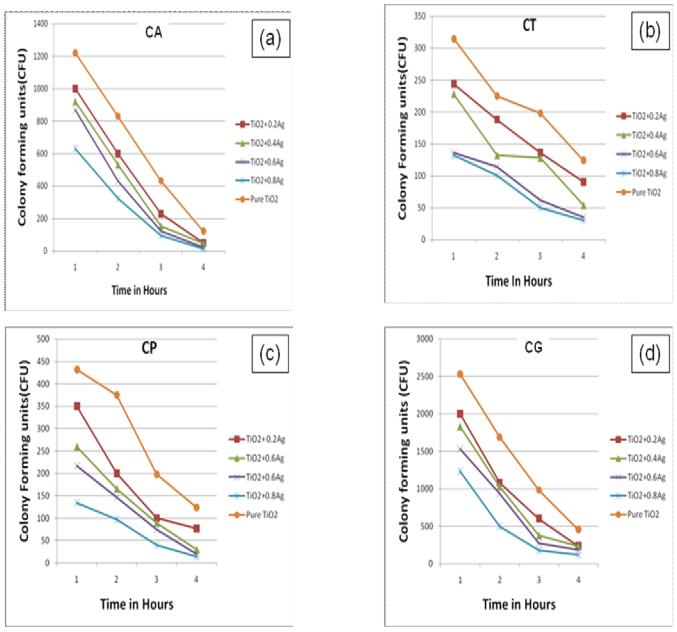

Fig 4: Colony forming units Vs time of exposure of nanostructured thin films of pure TiO_2 and TiO_2 with different concentrations of Ag as additive by cost effective sol-gel process annealed at 400°C for 1h in air ambient for the four fungal species a. *Candida Albicans* (MTCC-1637) ***(CA)*** *b. Candiada Glabrata* (MTCC 3019) ***(CG)*** *c. Candida Tropicalis* (MTCC-184) ***(CT)*** *d. Candida Parapsilosis* (MTCC-2509) ***(CP)***

All the bacterial species showed consistent susceptibility to TiO_2:Ag thin films namely *Staphylococcus faecalis* ***(SF)*** *(NCIM-2604). Staphylococcus epidermidis* ***(SE)*** *(NCIM-2493), Staphylococcus aureus* ***(SA)*** *(NCIL-2122), Bacillus subtilis* ***(BS)****(NCIM-2549* as shown in Figure 5a-d,

respectively. As the concentration of silver is increased the susceptibility also increased with all the bacterial species. *Staphylococcus aureus (NCIL-2122)* showed a drastic fall in count when compared with pure TiO_2 film (Fig-5c). It also showed considerable decrease in microbial count when the concentration of Ag is increased. Whereas other species have shown less change. Among the four species of microorganisms used *Staphylococcus epidermidis (NCIM-2493)* was found to be more resistant, but it showed drastic decrease in number once the concentration of Ag was increased from 0.6% to 0.8% (Fig-5b). However, it is worth noting that the surface finish of the highest Ag containing samples was very rough showing a large amount of pitting and consequently those coatings would not be effective antibacterial films. On the other hand, bacterial adhesion tests using *Staphylococcus faecalis (NCIM-2604)* showed a consistent reduction in number of colonies when the concentration of Ag is increased. Staphylococcus faecalis (NCIM-2604) showed consistent reduction in number when the concentration of Ag is increased in the base matrix of TiO_2 films.

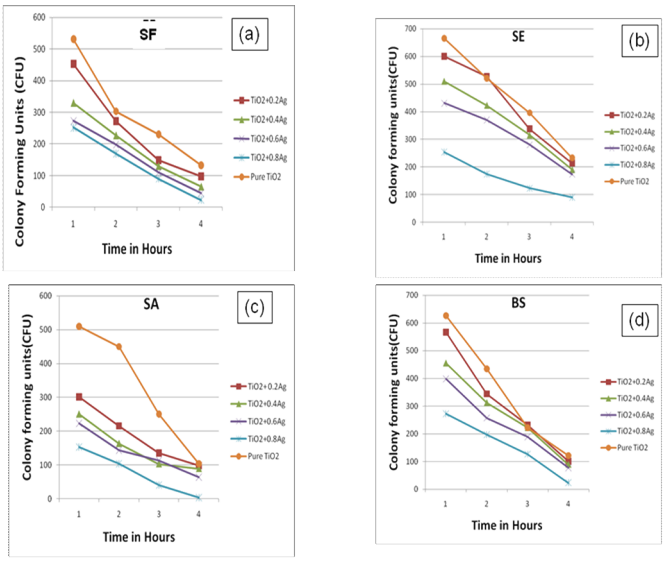

Fig 5: Colony forming units Vs time of exposure of nanostructured thin films of pure TiO_2 and TiO_2 with different concentrations of Ag as additive by cost effective sol-gel process annealed at 400°C for 1h in air ambient for the four bacterial species a. *Staphylococcus Aureus (NCIL-2122) (SA) b.Staphylococcus Faecalis (NCIM-2604) (SF)c. Bacillus Subtilis (NCIM-2549) (BS d. Staphylococcus Epidermidis (NCIM-2493) (SE)*

5. CONCLUSIONS

In this present investigation, we have systematically studied the antifungal (*Candida albicans* (MTCC-1637), *Candida tropicalis* (MTCC-184), *Candida parapsilosis* (MTCC-2509), *Candida glabrata* (MTCC 3019) and antibacterial properties of 4 species namely (*Staphylococcus faecalis (NCIM-2604). Staphylococcus epidermidis (NCIM-2493), Staphylococcus aureus (NCIL-2122), Bacillus subtilis (NCIM-2549)* each using pure TiO_2 and TiO_2 with Ag as additive by using simple and cost effective sol-gel process. The films have been deposited on glass and Si substrates and their structural, morphological properties have been investigated by employing standard spectroscopic techniques such as X-Ray Diffraction and Scanning Electron Microscopy, respectively. The test for antifungal and antibacterial has been carried out by using drop test method. It is evident from the results that with increasing Ag concentration the antimicrobial activity increases. The results are quite encouraging to apply for practical applications. Experiments are under progress to coat the best films on to the TiAlV alloys. As infection is one of the common clinical problems that cause high rates of death in various surgical operations, thin films coated on the surgical instruments or implants is a useful method to reduce such infections during the operation period.

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